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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Reported here is work performed during the first month of the above referenced program directed towards characterizing an advanced biosensor technology compatible for the real-time indirect optical detection of amino acids as typified by L-phenylalanine, L-dopa, L-tryptophan and related compounds. The technical strategy involves the indirect optical detection of amines and amino acids by monitoring changes induced in the visible spectra of immobilized chromagenic acyclic polyether host molecules to which the amine or amino acid becomes associated. Syntheses are currently proceeding for preparation of chromagenic acyclic polyether host mole-		

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only
cules into which selected amino acids will become incorporated, resulting in induced color changes, detectable when these molecular species are immobilized onto a fiber optic. Design and fabrication of synchronous sample-and-hold circuitry for detection of small optical changes from the fiber optic has largely been completed. (AW)
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ADVANCED BIOSENSORS FOR AMINO ACID DETECTION

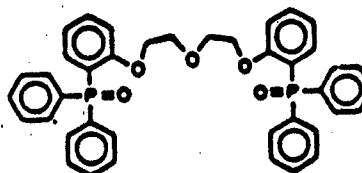
for the report period October 1 - 31, 1989

INTRODUCTION

This research effort is directed towards the identification, synthesis and application of organic molecules, which when coupled with optical waveguide technology will lead to the development of optical waveguide sensors (OWS) suitable for detection of amino acids and biogenic amines (i.e., dopamine). As an initial class of molecules for amino acid (amine) binding, we have chosen the so-called pcddands shown in I and II.



I



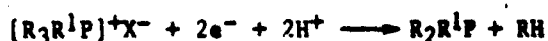
II

The use of acyclic polyethers such as (I) or (II) as molecular hosts for amine or amino acid binding have several attractive features including:

- the relative synthetic ease with which such molecular substrates may be prepared.¹
- the ability to tailor the host molecular substrate by varying either (or both) the polyether backbone or endgroups.
- the ability to incorporate into the polyether backbone a chromophore whose wavelength maxima may be sensitive to being shifted following binding with an appropriate cation species (i.e., an organic ammonium cation).²
- previously demonstrated and relatively rapid reversible binding kinetics afforded by these amine and amino acid hosts by the flexible polyether backbone.¹

Selection of (I) as a molecular substrate host for amino acids was prompted by previous work which has shown that inclusion of quinoline terminal groups onto acyclic polyether backbones can result in hosts possessing good stability for complexation with organic ammonium cations.³ Subsequent work has shown that quinoline containing acyclic polyether (I) possesses high selectivity for binding, transport and release of amines and amino acids across an aqueous electrolyte/CHCl₃ liquid membrane/aqueous electrolyte cell⁴ when (I) is present dissolved in the center chloroform layer. For example, (I) transports phentermine (C₆H₅CH₂C(CH₃)₂NH₂) or norephedrine (C₆H₅CH(OH)CH(CH₃)NH₂) respectively 170 or 62 times as rapidly across a CHCl₃ liquid membrane than either K⁺ or Na⁺. A similar structure, II, has also shown promise as an amino acid (amine) transport agent. Here the quinoline group is replaced by a phosphine oxide group such as triphenyl phosphine oxide, which is known to possess high formation constants for hydrogen bond formation⁵ which, when incorporated into an ap-

appropriate molecular structure, would be expected to provide an anchoring site for organic ammonium cations. This has been previously demonstrated⁶ for (II) which, when dissolved in CHCl_3 , was found effective for extracting phenylethylamine ($\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{NH}_2$) and di-phenylglycine ethyl ester ($\text{C}_6\text{H}_5\text{CH}(\text{NH}_2)\text{COOCH}_2\text{CH}_3$) from 0.2N aqueous perchloric acid solutions. In this case, some extraction selectivity was observed in that neither methylamine, ethylamine, propylamine or n-butylamines were removed from the aqueous electrolyte. The use of phosphine oxides as terminal groups could also, in principle, be used to selectively discriminate between di-isomers, since chiral phosphines can be conveniently prepared by a method of alternating electrocathodic cleavage of the P-C bond followed by quaternization,⁷ i.e.,

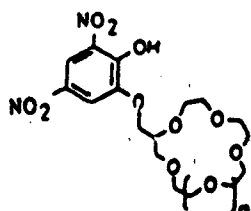


In addition to molecular recognition and selectivity, a host substrate molecule to be incorporated as a sensitive surface in a chemical biosensor should ideally also exhibit reversibility to the analyte of interest. Acyclic polyethers in general have been shown to exhibit more rapid reversible kinetics for complexation than crown ethers.¹ This property has been attributed to the flexible nature of the acyclic polyether backbone. Thus, as previously discussed, the ability of acyclic polyethers such as (I) to bind an amine, transport the amine across a CHCl_3 liquid membrane and then release the amine into an aqueous reservoir^{4,8} suggests that sensors incorporating similar acyclic polyethers as sensitive surfaces would exhibit reversible responses. Optical detection of bound amino acid (amine) analytes will be performed by attaching a chromophore to the acyclic polyether backbone.

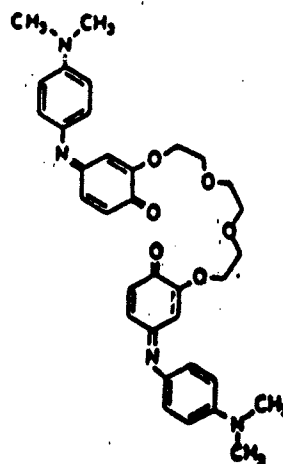
Recent work has shown that chromophoric groups can easily be incorporated into crown ethers to give ion selective colorimetric reagents.^{9a,9b} For example, when a 1,2-dichloroethane solution of (III) is brought into contact with an aqueous solution containing Na^+ species, the organic phase turns from yellow ($\lambda_{\text{max}} = 273\text{nm}$) to violet ($\lambda_{\text{max}} = 430\text{nm}$).¹⁰ Similar wavelength shifts have also been reported for acyclic polyethers such as (IV).^{9a} In host substrates (III) and (IV), complexation with a metal cation has been shown to induce an electron density shift into or out of the chromophore, thus perturbing the chromophore wavelength maxima. This approach can be applied to the indirect colorimetric determination of amino acids. Replacement of the central oxygen atom of (I) with a nitrogen atom may provide an additional hydrogen bonding site for the organic ammonium cation. When the nitrogen atom is introduced as part of an organic dye chromophore, hydrogen bonding by the amine or amino acid cation will be expected to influence electron density at the nitrogen atom, thus shifting the wavelength maxima of the chromophore which may be detected when incorporated onto a fiber optic probe.



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IV

Research progress performed to date will now be discussed.

RESULTS

Task 1 Syntheses of Chromagenic Polyether Host Molecules

Work has been initiated on the synthesis of chromagenic polyether host molecules starting from the precursors p-nitroaniline and 2-chloroethoxy ether. Initial syntheses proceeded using 11ml (.112 moles) of 2-chloroethoxy ether in 50ml DMF to which was added 6.907g (0.05 moles) of p-nitroaniline followed by refluxing overnight. However, subsequent gas chromatographic (GC) analysis of the reaction mixture using a Porapak Q column at 180°C showed essentially the same amount of 2-chloroethoxy ether as in the starting mixture. Thus, no reaction was promoted. Since resonance effects are important in aromatic amines and since p-nitroaniline is an extremely weak nucleophile, the lack of reactivity in the present system was probably due to low basicity of the amine function which made attack at the C-Cl bond unlikely. Following this

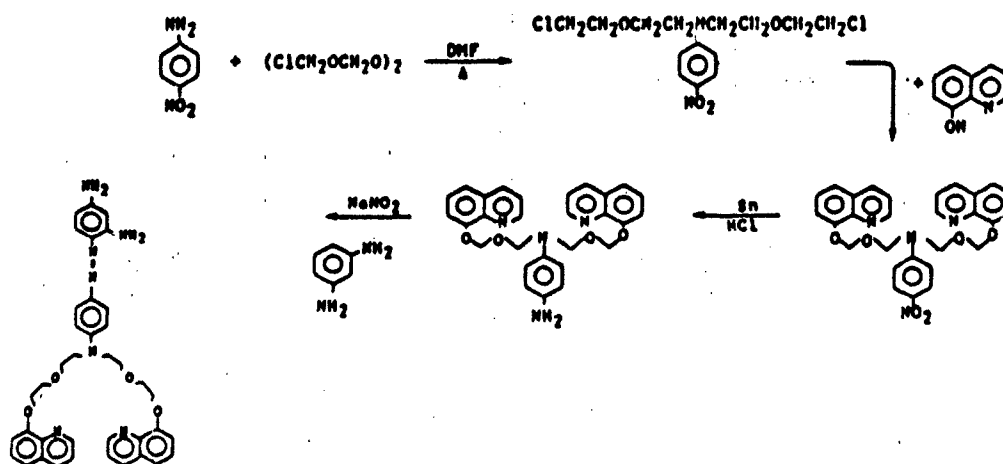
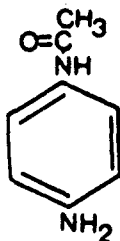


Figure 1. Summary of synthetic steps to be pursued in this program for preparation of chromagenic acyclic polyether hosts.

analysis we have now selected the aromatic amine 4-aminoacetanilide



as our precursor.

Here the acetamide group located para to the amino acid group would be expected to donate electron density to the latter, thereby promoting the desired reaction. Furthermore, the acetyl part of the protecting group would be available for convenient later removal via acid hydrolysis. The above reaction has been performed using 7.509g of 4-aminoacetanilide instead of 4-nitrotoluene. After heating at reflux for 6 hours, the reaction was stopped and analyzed using thin layer chromatography using silica gel plates and hexane, resulting in the identification of a new reaction product. Subsequent GC analysis showed a reduction (~20-40%) in the original 2-chloroethyl-ether peak. Currently reaction products are being separated using column chromatography on alumina with petroleum ether as the liquid phase. These products will then be subjected to appropriate analysis.

Task 2 Determination of Wavelength Absorption Maxima for Host Compounds and Wavelength Shifts When Exposed to Amino Acid Containing Solutions

No work has been performed on this task over the first 30 days of this program.

Task 3 Fabrication and Evaluation of Chromagenic Hosts in Cellulose Acetate Membranes Immobilized on Fiber Optic Probes for Amine and Amino Acid Analysis

The objective of this task is to characterize the performance and detection limits of chromagenic hosts immobilized in cellulose acetate on either the outside of an unclad optical fiber or onto the tip of a fiber optic probe for optical detection of amines and amino acids in aqueous solution.

Work performed during the first months of this program has emphasized development and fabrication of detector electronics. To this end an LED/photodetector arrangement along with associated electronics is being fabricated in order to provide high sensitivity measurements of optical changes along the optical waveguide sensor.

As seen from the schematic diagram (Figure 2), the transmitter circuit is based around a 555 timer arranged in a basic astable circuit with an output of about 14kHz (2/3 on, 1/3 off). The output of this is connected to the base of the power transistor D44HS which is used to drive the super bright LED. The LED, which has a peak emission at 660nm with a luminous intensity of 5000mcd, is coupled to a 1000µm fiber optic cable leading to the optical sensor and reference circuits.

The receiver is designed around two independent circuits, the first being the signal channel and the second the reference channel; both circuits are very similar. In these circuits the light from the sensor cable and the reference cable are coupled into two Sharp BS530UV photodiodes. As seen

from Figure 3, this detector has a very wide range of sensitivity, 200-1150nm with 500-900nm falling above 80% peak sensitivity. The use of signal and reference channels along with the use of sample and hold circuitry was chosen to provide low background drift and high signal to noise capability.

The photodiode is connected to the MOS/FET inputs of a CA3140A op. amp. The output is then fed through a DC blocking capacitor into an OP07 op. amp. circuit to further amplify the signal. This amplified signal is then fed into a SMP81 sample and hold circuit. The S/N signal is derived from the timer chip 555, hence the detection will be synchronous, allowing low levels of light changes to be detected. The sample and hold output is fed into a RC filter to remove any transients. In the final stage the signal is amplified to give a voltage directly proportional to the light coming from the sensor.

The use of a reference signal channel compensates for the well known drift in LED output with temperature changes. As a consequence, the signal channel is subtracted from the reference channel, thereby producing a stable baseline signal.

In addition to the receiver/transmitter circuits, a digital data acquisition system (Figure 4) is being fabricated to acquire and process the sensor signal via software. This data acquisition system consists of an analog to digital converter, memory, and microprocessor.

The microprocessor is an Intel 8052AH BASIC with built-in BASIC computer language, and an EPROM programmer, which can easily be attached to a PC such as the IBM/XT for data transfer. With 32K RAM + 8K EPROM, data will be taken during a given experiment and later analyzed on board or transferred to the IBM/XT for further analysis using standard software packages.

The analog to digital conversion is performed using a National Semiconductor ADC1205CGJ chip which has a resolution of 12 bits plus sign and a conversion time of 100µs. Depending on the voltage window being sampled, resolution ranges from 1.2 to .12mV (Table 1) will be achievable.

Finally, the microprocessor will also be wired to an alpha-numeric LCD module to monitor the photo voltage and other relevant information.

Initial operation of the prototype consists of zeroing the signal and reference channels while the optical sensor is "turned off". Once zeroed, the unknown chromagenic host molecule will be introduced into the system and the signal channel adjusted for signal optimization. This will then be stored into the data acquisition system for subsequent processing.

Table 1. Voltage window/resolution characteristics calculated at ERI for the natural semiconductor ADC1205CGJ chip.

<u>Reference Voltage</u>	<u>Resolution</u>
5.0 V	1.2mV
4.5 V	1.1mV
4.0 V	1.0mV
3.5 V	0.85mV
3.0 V	0.73mV
2.5 V	0.61mV
2.0 V	0.49mV
1.5 V	0.37mV
1.0 V	0.24mV
.5 V	0.12mV

Testing the receiver/transmitter circuits fabricated during the first month of this program was performed by directly placing a fiber optic cable between the LED and photodetector, with the reference and signal channels tied together for comparison. Viewing the signals on a Tektronics scope, voltage gains of about 25 for the signal channel and about 15 for the reference channel were observed. The final output signal was DC with a small AC component on top of it. Further filtering is expected to remove this AC component.

Optimization of the fiber optic electronic circuitry for amino acid detection will be expected completed during the next month.

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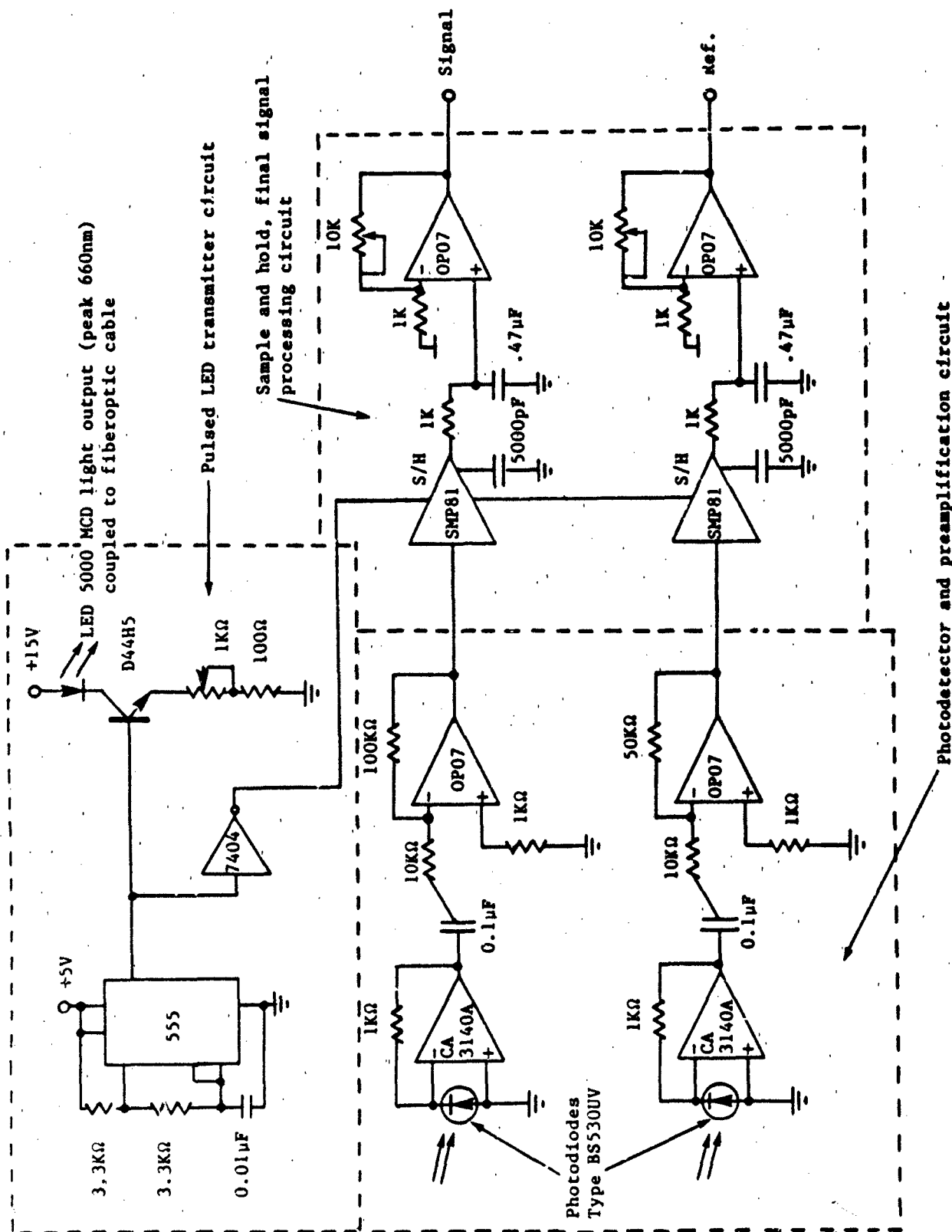


Figure 2. LED/Photodetector circuit for optical waveguide sensor.

Spectral Sensitivity

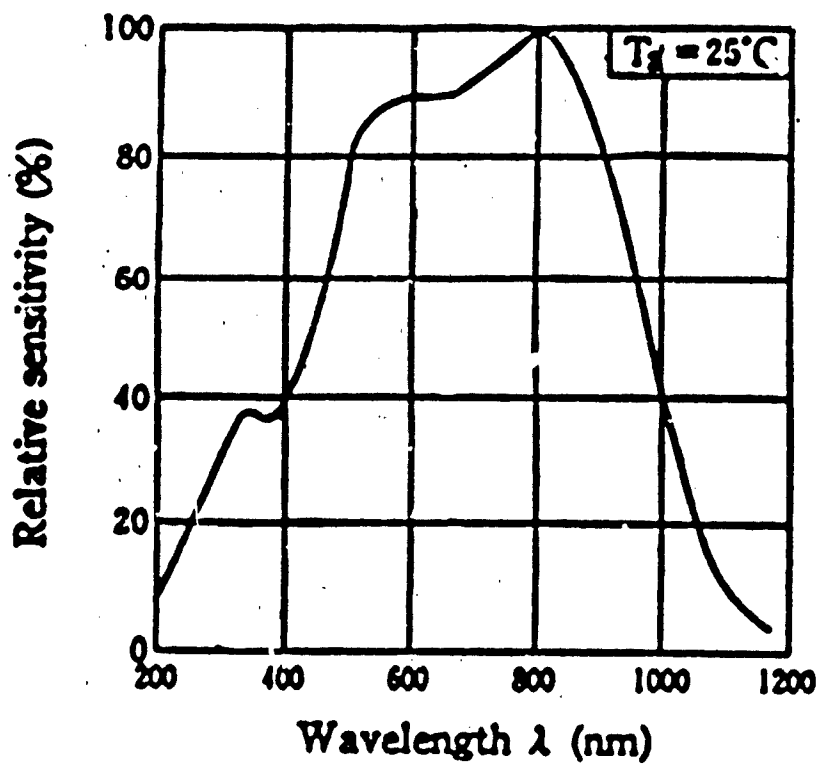


Figure 3. Spectral sensitivity of the Sharp B5530UV photodiode.

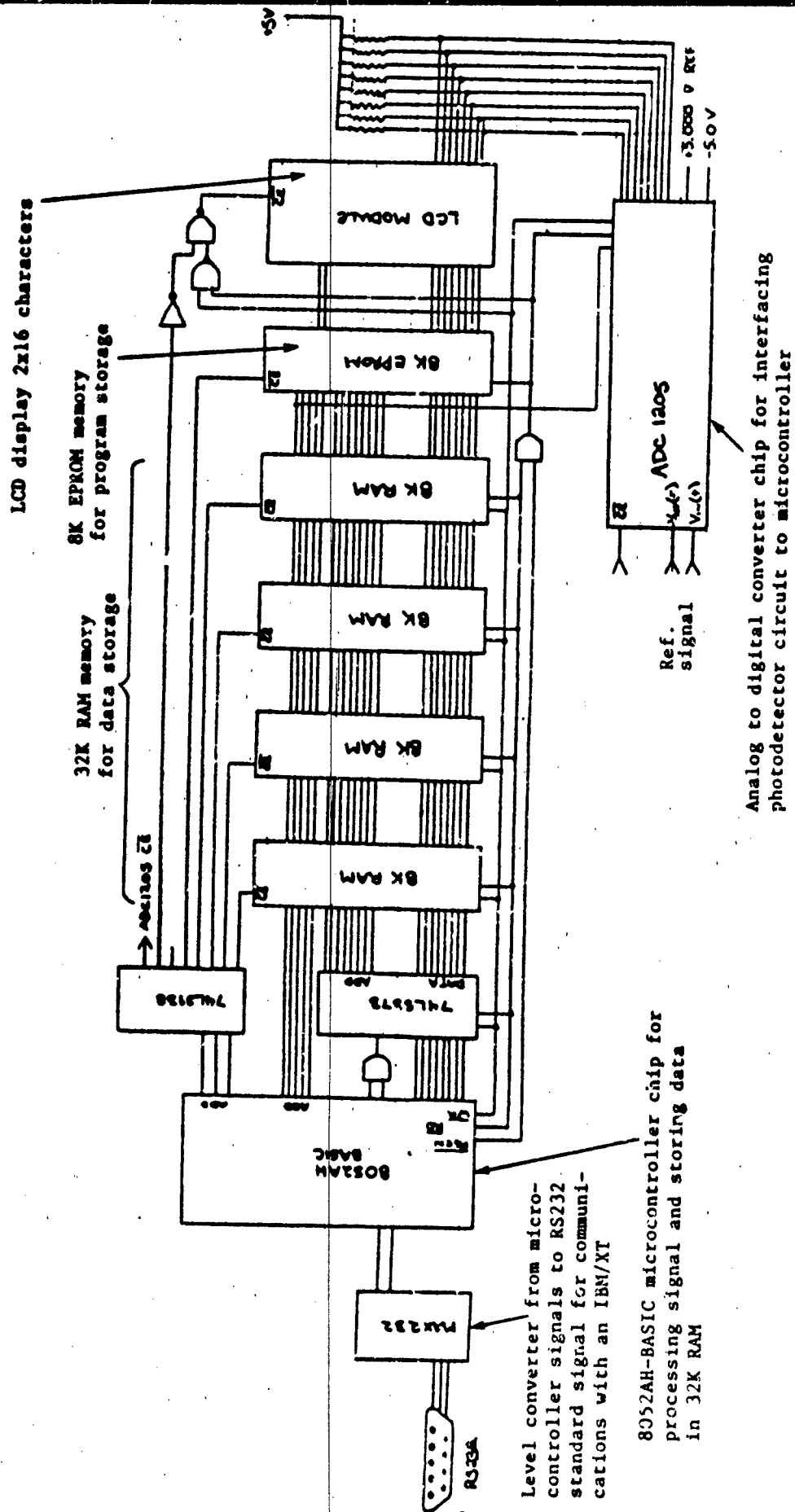


Figure 4. AD/DA converter circuitry for digital data acquisition.